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Effects of Ethrel® as Ripening Treatment for Cacao (*Theobroma cacao* L.) Pods

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ABSTRACT

Ethrel®, which contains ethylene, is used as a fruit ripening treatment. However, no information is available on its application as an artificial ripening agent for cacao pods. This study aimed to evaluate the effects of varying ethephon concentrations (0, 1000, 2500, and 5000 ppm) on cacao ripening in a completely randomized design with three replications. Unripe cacao pods, containing fully developed embryos, were utilized in this study. Cacao pods treated with 5000 ppm ethephon ripened five days earlier than untreated samples. In 1000 to 2500 ppm ethephon treatments, the day count required for ripening was three days after treatment (DAT). By 7 DAT, 100% of cacao pods treated with 2500 to 5000 ppm ethephon had ripened. The control treatment had the lowest percentage of ripened pods (50%) at 7 DAT. Total soluble solids (TSS) and titratable acidity (TA) of ripened cacao pods at 7 DAT were not altered substantially in response to ethephon concentrations. The highest TSS/TA ratio (29.13) was recorded in cacaos treated with 2500 ppm ethephon. In contrast, untreated cacao pods had the lowest TSS/TA ratio (23.49). Our findings suggest that ethephon can be used as a plant growth regulator (PGR) for cacao growers to accelerate the ripening of cacao pods.

Introduction

Using artificial agents, such as ethephon, to induce or accelerate fruit ripening has become prevalent in serving commercial purposes. Ethephon (2-chloroethylphosphonic acid) is an ethylene-releasing compound that has applications as preharvest and postharvest treatments to accelerate fruit ripening. Preharvest application of ethephon to synchronize fruit ripening within the plant has been found effective on coffee (Crisosto et al., 1991; Crisosto et al., 1992; Masasirambi et al., 2010), grapes (Farag et al., 2012; Silveira et al., 2016; González et al., 2018), durian (Wiangsamut et al., 2021), blueberry (Wang et al., 2018), fig (Sedaghat et al., 2023), sour cherry (Khorshidi

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and Davarynejad, 2010), plum (El-kady et al., 2014; Cocco et al., 2022) and pineapple (Hepton, 2003; Valleser, 2019).

As a postharvest treatment, ethephon has positive effects on the ripening of mango (Nair and Singh, 2003; Lacap et al., 2023), citrus (Morales et al., 2020), banana (Zenebe, 2015; Maduwanthi and Marapana, 2019; Timilsina and Shrestha, 2022), fig (Abdullah et al., 2022), pear (Dhillon and Mahajan, 2011), avocado (Rosas-Flores et al., 2020) and carambola (Le and Pham, 2018).

Before cacao pods ripen, the seeds reach physiological maturity. According to Niemenak et al. (2010) and Bridgemohan et al. (2016), this stage of cacao pod growth is designated as Biologische Bundesanstalt, Bundessortenamt, and Chemical Industry (BBCH) 79. The embryos fully develop at this point, and the growth of external dimensions on the fruit reaches 90% of the final size (Niemenak et al., 2010; Bridgemohan et al., 2016). Within the tree, cacao ripening is identified by a change in the color of the husk over 20-30 days (Niemenak et al., 2010; Bridgemohan et al., 2016). Unripe cacao pods contain seeds, which are difficult to extract and unsuitable for fermentation practices due to insufficient initial pulp fluid. Thus, only fully ripe pods should be picked from the tree (Bueno et al., 2020).

Based on our knowledge, no information exists on de-greening unripe cacao pods using ethephon as a treatment. When the cacao pods have reached physiological maturity at the BBCH 79 stage (Niemenak et al., 2010; Bridgemohan et al., 2016), we hypothesize that unripe cacao pods will ripen when subjected to ethephon treatment. In our opinion, there are several advantages to the artificial ripening of cacao using ethephon.

First, plant nutrients meant for BBCH 79 cacao fruit expansion are translocated to the young pods by harvesting cacao pods at this stage. As a result, nutritional competition among pods and cherelles is avoided. Therefore, this will indirectly reduce costs in fertilizer application. Second, harvesting cacao at the mature unripe stage reduces the risk of disease infection of cacao pods in the field. The pathogen *Phytophthora palmivora* black pod rot (BPR) attacks cacao pods at all stages of development (McMahon and Purwantara, 2004). Cacao pods can be harvested at the mature unripe stage during BPR disease seasons to prevent the fruits from becoming infected in the field. Third, seed germination within overripe pods is avoided by harvesting pods at the BBCH 79 stage. Lastly, unintentionally harvested unripe mature pods by cacao growers can still be artificially ripened.

This study aimed to determine if cacao pods at the BBCH 79 stage would continue to ripen after being detached from the tree. The effects of different ethephon concentrations on cacao ripening and pulp quality were investigated.

Materials and Methods

This study was conducted in Lantapan, Bukidnon, Philippines in June 2023. The cacao pods in this investigation were harvested from a cacao plantation located at Bangcud, Malaybalay City, Bukidnon.

Healthy pods at the BBCH 79 stage (Niemenak et al., 2010) (Fig. 1) were harvested from 'BR 25' cacao trees. The experiment was arranged in a completely randomized design (CRD) with four treatments and three replications, comprising a total of 12 experimental units. Each experimental unit consisted of four cacao pods.

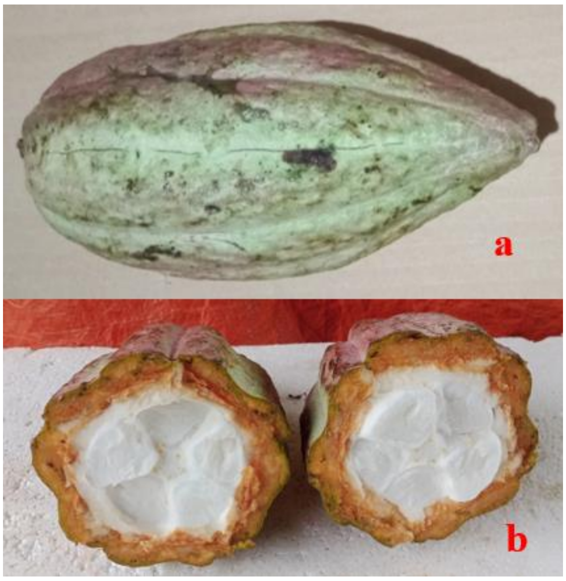

Fig. 1. Cacao pods at BBCH 79 stage used in the experiment; cacao shell appearance (a), intact cacao pulp from which beans are difficult to separate and collect from the husk (b).

Varying concentrations (0 ppm, 1000 ppm, 2500 ppm, 5000 ppm) of ethephon were prepared by mixing a required volume of Ethrel® (48% ethephon) with one liter of tap water. Different concentrations of ethephon solution were then applied as treatments to the cacao pods. Through a cotton ball, the treatment solution was swabbed onto the fresh wound of the cacao peduncle.

Cacao pods were then stored inside a room at ambient temperature and with an average relative humidity of 20%. Cocoa pod ripening was monitored until 7 days after the treatment applications (DAT).

Data collection

Days to ripening were determined by counting the number of days when 50% of the cacao pods from each experiment unit had ripened. Ripened pods (%) were assessed visually based on the color index for 'EET8' cacao as suggested by Garcia-Muñoz et al. (2021). Pods that appeared yellow to orange in color (color index 2-6) were counted and recorded according to the color index for 'EET8'. The data were expressed in percentages using the following formula:

$$Ripened\ pods\ (\%) = (Number\ of\ ripened\ pods)/(Total\ pods) \times 100$$

Pod weight was determined by weighing an individual cacao pod on a pre-calibrated weighing scale. This parameter was gathered before treatment application and at 7 DAT. Pod weight loss (%) at 7 DAT was also determined using the following equation:

$$Physiological\ loss\ of\ weight\ (\%) = (Initial\ pod\ weight - Pod\ weight\ at\ 7\ DAT)/(Initial\ pod\ weight) \times 100$$

Total soluble solids (TSS) of cacao pulp juice were measured using an AtagoTM handheld refractometer. Titratable acidity (TA) in this study was citric acid. Pulp juice (5 mL) from cacao was extracted and placed inside a beaker, and two drops of phenolphthalein solution were added. Titration then followed by adding a basic solution (0.1 N sodium hydroxide, NaOH) to the fruit juice until the color turned light red (Organization for Economic Cooperation and Development, 2005). The equation below was used for determining the TA (%):

$$TA\ (\%) = [volume\ (mL)of\ NaOH\ added \times 0.1\ (NaOH\ concentration) \times 0.064 \times 100]/(5\ mL)$$

Lastly, the TSS/TA ratio of cacao pulp juice was determined using the formula: TSS ÷ TA

Data gathered were subjected to the analysis of variance via Statistical Analysis for Agricultural Research (STAR 2.0.1) software (http://bbi.irri.org/products). Significant differences between mean values of treatment groups were separated using the least significance difference (LSD) test.

Results

Cacao pod ripening in response to varying ethephon concentrations

In the current investigation, unripe cacao (BBCH 79) pods continued to ripen even after they had been detached from the tree. Also, cacao pod ripening accelerated in response to higher doses (2500 ppm to 5000 ppm) of ethephon treatment (Figs. 2 and 3; Table 1). Ripening took two days after applying the highest concentration of ethephon (5000 ppm). However, it took three days for cacao pods treated with 1000 ppm and 2500 ppm ethephon treatments to ripen. In the control treatment (0 ppm ethephon), cacao pods needed seven days to ripen. Moreover, 100% of the cacao pods treated with 2500 to 5000 ppm ethephon ripened at 7 DAT (Fig. 2; Table 1). This was much higher than 1000 ppm, with only 58.53% of pods ripening at 7 DAT. The control treatment had the lowest percentage of pods that ripened (50%).

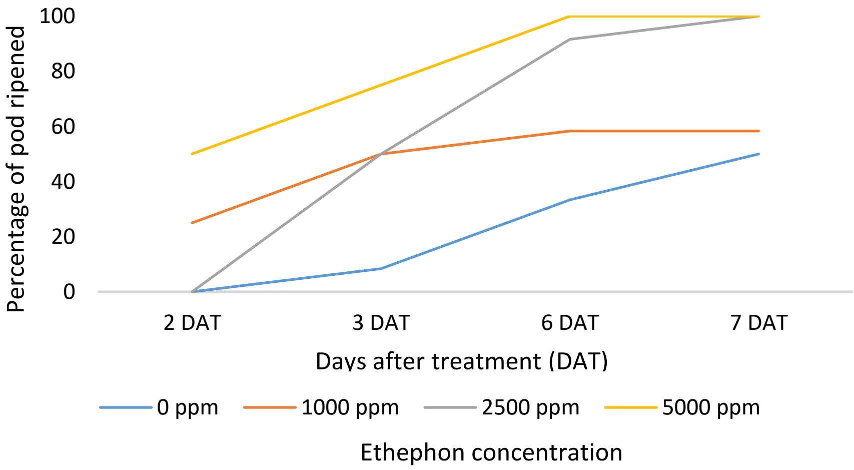

Fig. 2. Percentage of cacao pods ripened at 2, 3, 6, and 7 DAT as influenced by varying ethephon concentrations.

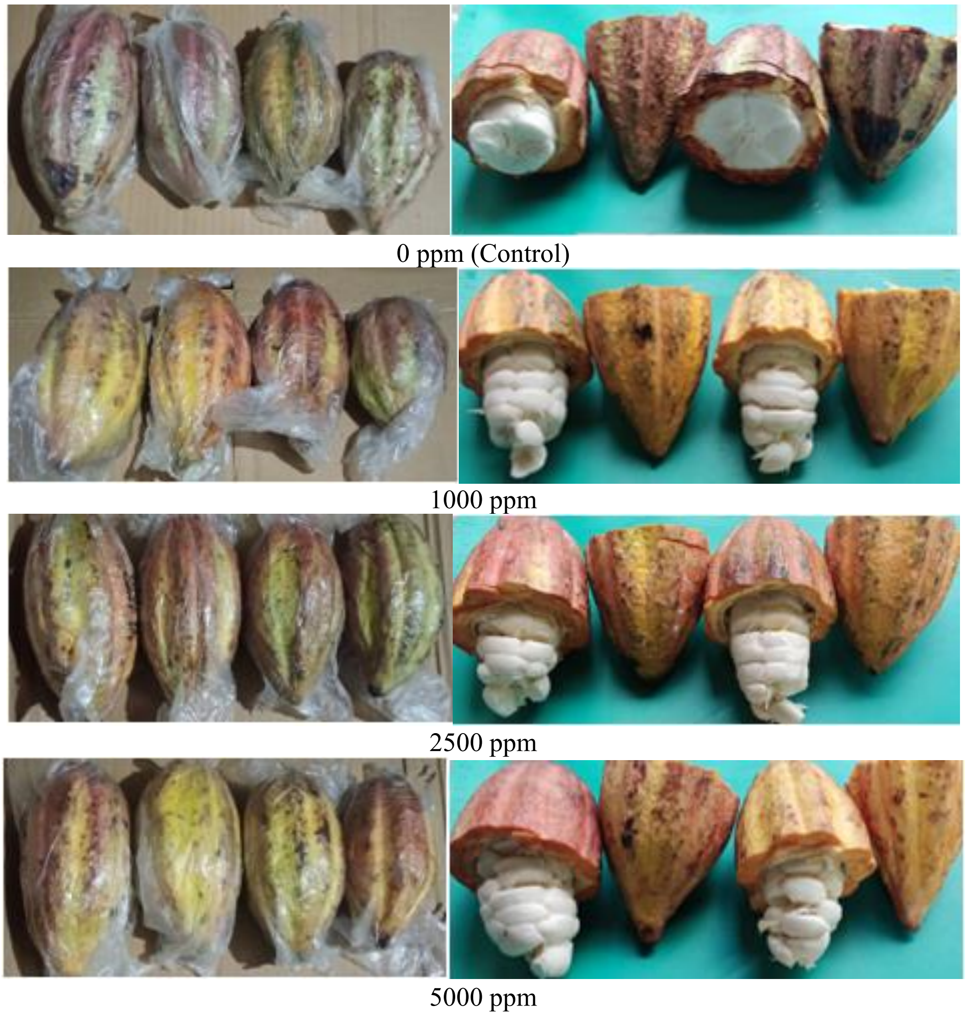

Fig. 3. Pod ripening (left photos) and pulp appearances (right photos) of cacao in response to varying ethephon treatments at 7 DAT.

Table 1. Number of days to cacao pod ripening and percentage of ripened pods at 7 DAT in response to varying ethephon concentrations.

Ethephon concentration (ppm)	Days to ripening[1/]	Ripened pods (%)
0	6.67^{a}	50.00^{c}
1000	3.00^{b}	58.53^{b}
2500	3.00^{b}	100.00^{a}
5000	2.00^{c}	100.00^{a}
Significance	**	**
cv (%)	7.87	15.38

[1/]- 50% of the pods show yellow to orange pigments.
**- Significance at 1% according to the Least Significance Difference (LSD) test. Mean values with the same letter as superscript are not significantly different.

Varying ethephon concentrations on the physiological loss of weight

The average initial weight of cacao pods in this study ranged from 452.42 to 464.83 grams (Table 2). The average weight of cacao pods in all treatment groups decreased at 7 DAT, when the pod weight ranged only from 442.75 to 448.42 g. It was noted that the 5000 ppm ethephon treatment caused the most significant weight reduction (3.74%) in cacao pods. Treatment with 2500 ppm ethephon caused the least significant weight reduction (2.57%), meaning that pod weight reduction was only minimal and did not vary statistically among the treatment groups.

Table 2. Pod weight of cacao pods before treatment application and at 7 DAT.

Ethephon concentration (ppm)	Pod weight (g)		Pod weight loss (%)
	Before treatment	7 DAT	
0	464.83	447.83	3.64
1000	464.25	448.42	3.40
2500	454.42	442.75	2.57
5000	454.50	437.50	3.74
Significance	ns	ns	ns
cv (%)	4.56	4.40	16.48

ns- not significant.

Effects of varying ethephon concentrations on TSS of cacao pulp

Ripened cacao pods in 2500 ppm ethephon treatment exhibited the highest TSS (15.53 °Brix) (Table 3). At 7 DAT, the TSS of the control sample was the lowest (14.47 °Brix). However, these TSS values were not significantly different from each other according to the LSD test. This result implies that the ethephon concentrations in this study did not alter significantly the TSS content in cacao pods.

Table 3. Total soluble solids, titratable acidity, and TSS/TA of cacao pulp at 7 DAT in response to various ethephon concentrations.

Ethephon concentration (ppm)	TSS (°Brix)	TA (%)	TSS/TA
0	14.47	0.6165	23.49[c]
1000	15.17	0.5760	26.43[b]
2500	15.53	0.5333	29.13[a]
5000	14.42	0.5504	26.22[b]
[1/]Mature ripe pods	15.50	0.5890	26.32
Significance	ns	ns	**
cv (%)	3.52	6.40	4.41

[1/]- harvested at the mature ripe stage (BBCH 81); average of three pods.
ns- not significant.
**- Significant at 1% LSD test. Mean values with the same letter as superscript are not significantly different.

Effects of various ethephon concentrations on the TA content in cacao pulp

When ripened, the pulp of cacao pods treated with 2500 ppm exhibited the lowest TA (0.5333%), whereas untreated cocoa pods exhibited the highest TA (0.6165%) (Table 3). However, these values were statistically comparable and were not significant according to the LSD test. This finding implies that various ethephon concentrations used in this study did not suppress TA content in cacao.

Effects of various ethephon concentrations on the TSS/TA ratio of cacao pulp

The ethephon concentration significantly affected the TSS/TA ratio in cacao (Table 3). The highest TSS/TA ratio in pulp juice was obtained from cacao pods in response to the 2500 ppm ethephon treatment, which was statistically different from cacao pods subjected to 1000 ppm and 5000 ppm ethephon treatments. The latter two had TSS/TA ratios of 26.43 and 26.22, respectively. Untreated cacao pods had the lowest TSS/TA ratio (23.49). In bananas, Timilsina and Shrestha (2022) reported that 750 ppm ethephon resulted in the highest TSS/TA ratio (31.51) in fruits. In pears, the chemical composition of fruits improved when 1000 ppm ethephon was applied (Dhillon and Mahajan, 2011).

Discussion

Ethephon is an active ingredient of Ethrel® and contains the stress hormone ethylene. Ethephon degrades when reaching the internal plant tissues, thus releasing ethylene, chlorate, and phosphate ions (Augusto and da Cunha, 2005). In most perishable crops, ethylene synthesis is prevented to increase the shelf-life and maintain the fresh appearance of commodities (Khandan-Mirkoh et al., 2020; Fazli and Ahmadi, 2024). However, on the positive side, ethephon has been used as a plant growth regulator (PGR) to ripen fruits and crops. It is often used for breaking down chlorophyll pigments to improve the aesthetic quality of fruits. To ripen fruits in commercial orchards, a liquid ethephon solution (i.e., Ethrel®) is applied to fruits that remain attached to the plants. This situation becomes a concern when applied to perennial trees because higher rates of ethephon (1000 ppm) can hinder tree growth (Crisosto, 1991). To prevent this problem, we used ethephon as a postharvest treatment for cacao pods. This method is similar to what has been applied to help fruits ripen artificially, such as in mango (Nair and Singh, 2003; Lacap et al., 2023), citrus (Morales et al., 2020), banana (Zenebe, 2015; Maduwanthi and Marapana, 2019; Timilsina and Shrestha, 2022), pear (Dhillon and Mahajan, 2011), avocado (Rosas-Flores et al., 2020), and carambola (Le and Pham, 2018).

Our results suggest that ethephon treatment can hasten the ripening process in cacao pods harvested at the mature unripe stage (BBCH 79). This result agrees with previous findings by Lacap et al. (2023) that ethephon functioned as a ripening agent in 'Carabao' mango. At 1000 ppm ethephon treatment, the required timespan to reach marketability in 'Carabao' mango decreased from six days to only 3-4 (Lacap et al., 2023). Similarly, Rosas-Flores et al. (2020) reported that avocado fruits can ripen two days earlier in response to ethephon. Rates of ethephon at 1000 ppm and 500 ppm were optimal for cultivars 'Hass' and 'Mendez,' respectively (Rosas-Flores et al., 2020). In bananas, 750 ppm of ethephon was the lowest effective concentration to ripen the fruits (Timilsina and Shrestha, 2022).

In our present study, cacao pods exhibit changes in rind color, and the pods were softened (based on the feel method) in response to ethephon treatment. This change allowed the seeds to separate from the mesocarp, making these seeds easier to extract and collect. Unlike the unripe pods in the control treatment, seeds from ripened pods (Fig. 4) contain the moisture needed for fermentation and subsequent postharvest processing. This condition implies that the respiration rate in cacao pods increased in response to the ethephon treatment.

Cacao pulp contains 82-87% water and 10-15% sugars (Afoakwa, 2016). Sugars are broken down into simpler forms during respiration, producing CO_2, water, and energy. Regardless of the ethephon concentrations applied, this breakdown causes a rise in the moisture content of the pulp and softening of the ripened cacao pods. Our findings on this parameter, however, differed from what has been reported by Laylah et al. (2023) on the Sulawesi 2 (S2) cocoa clone, implying that there is a varietal variability in cacao postharvest ripening.

Fig. 4. Extracted seeds from unripe (a) and ripened (b) cacao pods at 7 DAT. Note the moisture content of cacao pulp from ripened cacao pods.

Ethephon treatments did not affect the weight loss of cacao pods at 7 DAT in our experiment. At 7 DAT, weight loss was minimal, ranging from 2.57 to 3.74%. Our results differ from a report by El-kady et al. (2014) on plums, where ethephon treatment significantly contributed to fruit weight reduction. As a common packing house practice, pre-harvest bags should be removed from fruit crops that must be sold fresh to apply appropriate postharvest treatments. In our study, however, the clear cellophane bag, used as a pre-harvest covering for the pods, was not removed because cacao needs to be sold as a fresh product. Cocoa beans inside the pods were the goal of this research. In postharvest, various packaging materials can minimize water loss in papaya (Azene et al., 2014) and mango (Vilvert et al., 2022). Accordingly, in our study, the pre-harvest cellophane bag acted as a barrier that helped minimize water loss in the cacao pods.

In some fruit species, ethephon treatment significantly altered the TSS value. For instance, in mango (cv. ‘Kesar’) (Doke et al., 2018), banana (El-kady et al., 2014), and fig (Abdullah et al., 2022), ethephon treatment reportedly increased the fruit TSS. In contrast, ethephon treatment reportedly reduced TSS content in Japanese plums (Cocco et al., 2022) and sour cherries (Khorshidi and Davarynejad, 2010). In our study, the TSS of cocoa pulp was not significantly affected by ethephon treatments. Furthermore, the TSS contents of cacao pulp in our study are similar to the TSS contents of naturally ripened cacao pods (Table 3) and consistent with the findings of Oldham et al. (1997). This finding infers that cacao pods are harvestable at the unripe stage for as long as they reach physiological maturity (BBCH 79). Furthermore, varying ethephon rates did not suppress the TSS in cacao. Cacao pulp is an excellent microbial growth substrate. It has a moderate sugar content (10-15%) and a small citric acid content (0.4-0.8%) (Malaysian Cocoa Board, undated). The sugar concentration of cocoa pulp is influenced by genotype. Oldham et al. (1997) stated that Amelonado, Amazonia, and Hybrid had 14.32%, 15.37%, and 14.82% initial pulp sugar, respectively.

The effect of ethephon on fruit TA is influenced by the fruit species and the ethephon rates applied. Fruits such as blueberry (Cocco et al., 2022), fig (Abdullah et al., 2022), and mango (Doke et al., 2018), when subjected to ethephon treatment, showed lower TA than the untreated ones. In kiwi fruits, the TA of juice was higher in fruits treated with 500 ppm ethephon than the fruits with 1000 ppm ethephon treatment (Bal and Kok, 2007). In our present study, various ethephon treatments did not affect TA content (citric acid) in cocoa pulps. Our result is similar to that of Khorshidi and Davarynejad (2010) that ethephon treatment had no significant effect on TA in sour cherry fruit. However, the TA content in cacao pulps in our study, regardless of treatment, was within the expected range (0.4-0.8%) published by the Malaysian Cocoa Board (undated). This result indicates that the typical TA value of cacao pulp required for fermentation is attainable as cacao pods harvested at the BBCH 79 stage can ripen in response to ethephon rates. In addition, varying ethephon rates showed no undesirable effects on TA.

The highest TSS/TA ratio in pulp juice occurred in cacao pods treated with 2500 ppm ethephon. However, untreated cacao pods had the lowest TSS/TA ratio (23.49). These findings indicated that ethephon treatment had no adverse effects on the TSS/TA ratio in cocoa pulp juice. TSS and TA are crucial for the cocoa fermentation process, influencing it significantly because they affect the development of yeast and bacteria involved in the process while regulating the biochemical changes that occur inside the beans (Rojas et al., 2020). Ethephon treatments positively affect the TSS/TA ratio in fruits, and the effective dose varies between fruit species. In banana fruits, Timilsina and Shrestha (2022) reported that 750 ppm of ethephon resulted in the highest TSS/TA ratio (31.51). Similarly, Abdullah et al. (2022) reported that the TSS/TA ratio of fig fruit treated with 1500 ppm ethephon was significantly higher than in fruits from other treatments. In pears, the chemical compositions of fruits were better when 1000 ppm of ethephon was applied (Dhillon and Mahajan, 2011). In the 'Kensington Pride' mango, fruits dipped in 500 ppm ethephon for five minutes showed a higher TSS/TA ratio (Nair and Singh, 2003).

Conclusion

When harvested at the BBCH 79 stage, ethephon (2500 to 5000 ppm) accelerated the ripening of ‘BR 25’ cacao pods. In our study, the ideal ethephon concentration for ripening 'BR 25' cacao pods at BBCH 79 was 2500 ppm. As a cacao ripening treatment, ethephon may find applicability to other locally available cacao varieties at the BBCH 79 and BBCH 80 stages. Also, we recommend analyzing the biochemical contents not measured in this study and sensory evaluations of cacao pulp and beans after ethephon treatments.

Acknowledgments

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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